Accepted Manuscript

Continuous flow thin film microfluidic mediated nano-encapsulation of fish oil

Shan He, Nikita Joseph, Xuan Luo, Colin Raston

PII: S0023-6438(18)31133-2

DOI: https://doi.org/10.1016/j.lwt.2018.12.066

Reference: YFSTL 7727

To appear in: *LWT - Food Science and Technology*

Received Date: 13 August 2018

Revised Date: 15 November 2018

Accepted Date: 24 December 2018

Please cite this article as: He, S., Joseph, N., Luo, X., Raston, C., Continuous flow thin film microfluidic mediated nano-encapsulation of fish oil, *LWT - Food Science and Technology* (2019), doi: https://doi.org/10.1016/j.lwt.2018.12.066.

Continuous flow thin film microfluidic mediated nano-encapsulation of fish oil

Shan He[1, a ^*], Nikita Joseph [a ^], Xuan Luo [a], Colin Raston [a*]

[1] *Department of Food Science and Engineering, School of Chemistry Chemical Engineering, Guangzhou University, 510006, China*

[a] *Institute for NanoScale Science and Technology, College of Science and Engineering, Flinders University, Bedford Park, SA 5042 Australia*

*Corresponding author:

Prof. Colin L. Raston, Tel: +61 8 82017958.

Email: colin.raston@flinders.edu.au

Associate Prof. Shan He, Tel: +61 401509763

Email: he0091@gmail.com

^ These two authors contributed equally to this work

Abstract

A facile process has been developed for the encapsulation of fish oil using a thin film vortex fluidic device (VFD) operating under continuous flow, allowing control over the size of the encapsulated particles which are spheroidal in shape with diameters ranging from 50 to 250 nm. This microfluidic platform simplifies the processing procedure of water-in-oil-in-water (w/o/w) encapsulation, as now a time and cost saving one-step process devoid of any organic solvents, in contrast to the conventional homogenization process which is inherently complex, involving multiple-steps and the use of organic solvents. Moreover, use of homogenization (as a benchmark to encapsulate fish oil) afforded much larger macro-particles, ranging in size from 2 to 4 μm. Smaller encapsulated particles fabricated using VFD processing could lead to improved absorption from fish oil. Overall, VFD processing provides a new alternative bottom–up approach to easy, scalable processing, without the need for organic solvents which are counterproductive in the food industry.

Key words: Vortex fluidic device; Encapsulation; Nano-particles; Continuous flow.

1 Introduction

Long chain omega-3 polyunsaturated fatty acids (PUFAs) are the major component in fish oil, and they are beneficial for human health, improving the fluidity and function of components of cell membranes, and thus enhancing cell metabolism（Li *et al*., 2015). PUFAs are however prone to oxidation, resulting in poor bio-availability to the body (Li *et al*., 2015). This is compounded by their lipophilic property imparting poor water solubility, and this can be counterproductive for commercial applications of PUFAs. Encapsulation

technologies and various colloidal delivery carriers can be used to overcome the bioavailability of omega-3 in our diet, while also allowing its improved controlled release.

The conventional method for oil encapsulation involves the use of a surfactant which forms a layer in stabilising the oil-water interface. These encapsulated particles are conventionally processed through high-energy methods, such as homogenization. The purpose of this is to break the bigger particles into smaller particles through mechanical shear force. Nazar, Hafeezullah & Shahzad (2010) homogenized whey protein isolate and beeswax, forming beeswax encapsulation particle ranging in size from 0.5 to 2.0 µm. Sacanna & Philipase (2006) used monodispersed latex to stabilize a water and oil encapsulation system, with the average diameter of the encapsulated particle ranging from 35 to 75 µm. Compared with "µm encapsulation", "nano-encapsulation" provides better characteristics for commercial use in foods, in providing higher absorption in the body and instantaneous release of flavour, and for use in injection medication (Li *et al*., 2015). However, it is difficult for conventional homogenization to generate particle-sizes at the nano-meter dimensions, unless operating the processing at high pressures, as reported by Mostafa, Jean-Rene, Marc-Antoine & Harivardhan (2018) who produced nano-crystalline suspensions under such conditions, approximately 200 nm in diameter. Moreover, the high cost of a high pressure homogenizer (approximately USD 150k) limits its application mainly to laboratory operations, rather than extending them into industry.

The vortex fluidic device (VFD), Fig. 1, represents a relatively new thin film microfluidic processing platform that has a plethora of diverse research and industrial applications (Britton & Raston, 2017). VFD processing technology emerged from research efforts focused

on the application of thin film microfluidics and thin film flow chemistry (Chen, Smith, Iyer, & Raston, 2014; Britton & Raston, 2017). It harnesses high shear forces, intense micro mixing, and high heat and mass transfer, overcoming mixing and heat transfer limitations of traditional batch processing (Britton *et al*., 2015). Processing capabilities of the VFD are rapidly growing, from small molecule synthesis through to processing functional materials for drug delivery, and manipulating single cell organisms (Britton & Raston, 2017). The VFD can be operated under a confined mode where a tube containing a finite volume of liquid is spun at high speed, or operated under continuous flow whereby jet feeds deliver liquid to the bottom of the tube or at positions along the tube, with the device operating otherwise under the same conditions. At high rotational speeds, the liquid in the tube forms a dynamic thin film with the thickness of the film controlled by varying the rotational speed, tilt angle of rotation, and the volume of liquid in the tube (Britton, Chalker, & Raston, 2015).

The utility of the VFD has been established for a number of chemical transformations, with control over reactivity and selectivity, and the ability to prepare complex molecules in a single pass. Dramatic enhancement of enzymatic reactions have been established, with an average seven-fold acceleration in their catalytic reactions, which arises from Faraday pressure waves within the thin film (Britton, Meneghini, Raston, & Weiss, 2016). Tethering enzymes to the surface of the tube has also been achieved, for the synthesis of complex molecules in a single pass (Britto & Raston, 2017; Britton, *et al.*, 2017). Then there are the remarkable results for materials processing under continuous flow, for example, in assembling fullerene C_{60} molecules into nanotubules (Vimalanathan, Chen, & Raston, 2017). The micron length nanotubules, with hollow diameters of 100 to 400 nm, are formed in the

absence of surfactant, and without the need for further downstream processing (Vimalanathan *et al*., 2017).

The 3 major VFD operating parameters are rotational speed, flow rate and tilt angle, and these determine the outcome of the high throughput processing. Therefore, the optimization of these 3 parameters is essential for VFD processing and changing them may significantly affect the processing outcome. For example, VFD processing is effective in converting sunflower oil to biodiesel at room temperature, with no saponification, and avoiding the conventional use of co-solvent or complex catalysts. When the flow rate increased from 1 mL/min to 5 mL/min, the percentage of conversion to biodiesel dropped from 100% to 80% (Britton & Raston, 2014). VFD processing is also effective in direct transesterification to produce biodiesel from wet microalgae biomass at room temperature. When the rotational speed is increased from 4,000 rpm to 8,000 rpm, the conversion efficiency of fatty acid methyl ester increased from 30% to 90% (Sitepu *et al.,* 2018). We were motivated to explore the aforementioned high shear forces and intense micro mixing in the VFD for encapsulation purposes. While the VFD has not been previously used for this purpose, it has been used for uptake of anti-cancer drugs in preformed vesicles of phospholipid mimics (Mo *et al*., 2015).

The purpose of the present study is to investigate nano-encapsulation of fish oil, which is rich in poly unsaturated fatty acids, under shear stress in the dynamic thin film in the VFD, in the presence of phospholipids, which act as stabilisers between the aqueous and non-aqueous phases. This VFD-mediated process was compared with the conventional homogenization process, and their encapsulation efficiencies calculated. This has led to

119 establishing a one-step, time-saving process for the formation of nano-encapsulated
120 particles, which is without precedent.
121
122 **2 Materials and Method**
123 Fish-oil enriched with omega-3 fatty acids was provided by Chemist Warehouse (Australia),
124 and the non-ionic surfactant 1-palmitoyl-2-oleoyl phosphotidylcholine (POPC) was obtained
125 from Sapphire Bioscience (NSW, Australia). Milli-Q water was used throughout the
126 preparations of encapsulated fish oil.
127
128 ***2.1: Preparation of nano-encapsulations***
129 Nano-encapsulations were formulated using fish-oil, as a bioactive ingredient, and a mixture
130 of liposome as surfactant, and water. Oil in water nano-encapsulations had a liposome to oil
131 ratio of 1:1 (w:w) with a concentration of the content of liposome and oil to total volume of
132 the suspension in 2 mg/mL. Briefly, 10 mL lipid, oil and water suspension was prepared
133 which contained 20 mg of fish oil, 20 mg of lipid, and 10 mL of water, affording a
134 concentration of lipid and oil at 2 mg/mL with the ratio of lipid to oil at 1:1 (w/w). This
135 suspension was premixed, and then introduced into the borosilicate glass tube (20 mm OD,
136 17 mm ID) in the VFD through jet-feeds. The VFD operational parameters were: rotational
137 speed of 8000 rpm, flow-rate 0.1 mL/min, tilt angle of 45^{o}. This condition was applied as the
138 optimal condition after optimization of speed (4,000 rpm to 8,000 rpm, Fig. S1, Fig. S4),
139 flow-rate (0.3 mL/min to 0.5 mL/min, Fig. S2, Fig. S4), and tilt angle (30^{o} to 60^{o}, Fig. S3, Fig.
140 S4). The device was operated at room temperature and the product processed in the tube
141 was collected and sonicated for 20 min. The traditional homogenization method was also
142 applied for comparison with the VFD processing. Here the same volume, concentrations and

ratio (10 mL lipid, oil and water suspension) was prepared which contained 20 mg of fish oil, 20 mg of lipid, and 10 mL of water, with the concentration of lipid and oil at 2 mg/mL and the ratio of lipid to oil at 1:1 (w/w) (as used in the VFD). This was then homogenized (homogenizer T25 digital ULTRA-TURRAX) at 13,500 rpm for 10 min at 25°C. Products processed by homogenization were also collected and sonicated for 20 min.

2.2: Particle Size

Nano-encapsulation particle size distribution and polydispersity index were determined at 25°C using dynamic light scattering (DLS) (Nano ZS90, Malvern instruments, Worcester, UK) operating with a He-Ne 633 nm wavelength laser and a detector angle of 173°. Three independent measurements were performed for each sample. The Malvern zeta sizer instrument measured the time dependent fluctuations of light scattered based on the particle sizes. Samples were too viscous to be analysed immediately using DLS, but this dropped with time, with the data recorded 20 min after VFD processing. Also noteworthy is that there was two phase-separation of sample using homogenization processing over 24 h. In contrast, two-phase-separation was not observed from samples generated using VFD processing.

2.3: Scanning Electron Microscopy (SEM)

Samples with nano-encapsulated particles were analysed using an Inspect FEI F50 SEM (PS216) instrument. The spot size was 2.0, voltage was 5.0 kV and magnification was 50,000. Sample preparation for SEM was as follows: 20 µL of as-prepared sample was drop casted on a silicon wafer and air dried overnight, followed by platinum sputter coating of a 2 nm thick layer, for then SEM imaging.

2.4: Transmission Electron Microscopy

The nano-encapsulated particles were analysed using transmission electron microscopy (TEM) (FEI Titan Themis 80-200). The sample preparation was as follows: 20 µL of sample was fixed on the carbon grids and left to air dry for 1 h, with the excess sample removed using blotting paper, followed by staining using 2 g/100 mL uranyl acetate solution. TEM images were then taken at 250× magnification under the voltage of 300 keV.

2.5: Atomic Force Microscopy

Samples with nano-encapsulated particles were analysed using atomic force microscope (AFM) (Nanoscope V, Multimode 2 SPM). Images were acquired using silicon probes in tapping mode in air with Scanner "E" from 500 nm^3 µm scan sizes. The sample preparation for AFM was as follows: 20 µL of 1: 100 diluted sample was drop casted onto silicon wafer and air dried overnight whereupon the samples were washed 3 times with Milli-Q water prior to recording the AFM images.

3 Results and Discussion

3.1: Formation of fish-oil in water encapsulations

Processing carried out in the VFD with the tube rotating at 8,000 rpm, using a flow rate of 0.1 mL/min, tilt angle 45^{o}C, and a temperature of 25^{o}C, afforded a solution that was distinctly different in appearance relative to encapsulation using conventional homogenization. The fish oil solution produced using VFD processing was clear (Fig. 2c), whereas the solution produced using conventional homogenization was milky (Fig. 2b).

After processing using both techniques, samples were sonicated for 20 min., prior to characterisation. Sonication alone (no VFD) effects the physical appearance of the different samples if it was applied for a long period of time. Adeyemi *et al.* (2017) advised that bath sonication has to be applied for a minimum of 30 min to have an impact on physical appearance of nanoparticle suspension. The shorter sonication time used in the present study, 20 min, was applied to avoid aggregation of particles, and thus the results from VFD processing and conventional homogenization can be directly compared.

The particle size in solution is one of the decisive factors for determining if the solution is transparent or otherwise. Honary, Ebrahimi & Hadianamrei (2014) reported that solutions change from milky to clear when the particle size is reduced from micrometres in dimensions to about 200 to 300 nm. A similar particle size encapsulation has also been reported (Betoret, Betoret, Rocculi & Rosa, 2015). Thus, previous studies are consistent with the different physical appearance between the encapsulation solutions in Fig. 2b and c. To further understand the origin of the difference, a number of imaging characterisation techniques were used.

3.3 Scanning Electron Microscopy (SEM)

SEM images of the particle are consistent with the results shown in Fig. 2. The conventional homogenization method produced particles approximately 3 to 4 µm in diameters (Fig. 3a). In contrast, the particles generated using VFD processing, Fig. 3b, are much smaller, in the nano-meter-dimensions, < 300 nm in diameter. We note that while this is similar to those generated using an expensive high-pressure homogenization system, the VFD is relatively inexpensive processing technology. Thus, VFD processing potentially represents a significant

cost saving approach for encapsulation at the nano-meter-dimensions, noting that the VFD is a relatively inexpensive device.

3.2 Dynamic Light scattering (DLS) technique

The polydispersity, and the hydrodynamic diameter of the particles generated using VFD processing and homogenization processing were determined using DLS analysis (Fig. 4), revealing similar polydispersity (0.27 for VFD process, 0.192 for homogenization process), and 20 to 250 nm particles with the majority of them distributed between 50 nm to 250 nm in diameter. DLS showed a similarity in diameters of the particles (approximate 50-250 nm, Fig.4) relative to diameters from SEM data (approximate 100-200 nm, Fig.3), for particles generated using VFD processing (hundreds of nm regime). The DLS measurement of particles generated using homogenization processing is different to that determined using SEM (Fig.3a) which were between 2µm and 4 µm. This not surprising given that homogenization samples were turbid, unstable, and not transparent, and for such samples DLS is expected to be problematic. The characteristics of the homogenization sample also explain its aforementioned two phase-separation over 24 h.

3.4: Transmission Electron Microscopy (TEM)

The internal structure of the fish oil particles encapsulated using a VFD was studied using TEM. For the image in Fig. 5, the sample was stained with a 2 g/mL solution of uranyl acetate. The oil phase, which has PUFAs with carbon backbones, takes up more of the uranyl acetate than the aqueous phase, and thus appears darker. The Image shows multiple w/o/w encapsulations in which water particles are trapped in the oil-phase. w/o/w encapsulations are formed when an oil phase, in the presence of surfactant, is rapidly

stirred or homogenized at high speed in an aqueous medium. Previous studies also report the formation of the w/o/w encapsulation system. Meng, Guang, Wei & Zhi (2003) used ethyl acetate as the organic solvent to form the w/o/w double encapsulation, and Herrmann & Bodmeier (1995) formed the multi-phase of w/o/w microspheres by applying a multiple encapsulation solvent evaporation technique. However, these earlier processes are complicated, with Meng *et al.* (2003) taking 5 steps and Herrmann *et al.* (1995) taking 3 steps to form the w/o/w encapsulation system, both using organic chemicals. In contrast, the VFD method is a one-step process, which is time-saving, and its use circumvents the need for using any organic solvents, and thus the overall process in is more environmentally friendly. Importantly, processing in the VFD simplifies the processing relative to previous methods for w/o/w encapsulation.

3.5 Atomic Force Microscopy (AFM)

The particles generated in the VFD were further studied using AFM, as shown in Fig. 6 (Bruker's AutoMET software analysis) with the height of the encapsulated particles estimated to range from 10 to 20 nm (Fig. 6), interestingly with a 10 nm height approximating to the dimension of two bi-layers of the self-assembled phospholipid, without any content inside the particles.

3.6 Volume of particles

DLS provides information on the hydrodynamic diameter of the particles, treating them as spherical particles, whereas any anisotropy in shape can come from imaging techniques. The 50 to 250 nm diameter of particles from DLS corresponds to volumes of the particles 65,500 nm^3 to 8,177,000 nm^3. TEM measurement established that a typical diameter of a

particle is approximate 200 nm. AFM measurement showed that the height of the particles as 10 nm and 20 nm, which corresponds to volumes between 314,000 nm^3 and 628,000 nm^3, and are within the volumes of particles established using DLS.

3.7 Scale-up VFD processing to produce encapsulated fish oil solution

The VFD is designed with the ability to operate under continuous flow for on-demand processing of large volumes of liquid. To establish this herein, we readily translated the processing of a 10 mL solution, as used for all of the above studies, to passing 100 mL of solution within 18 h through the device, with the solution found to have the same overall characteristics, including colloidal stability. The success of this trial demonstrated the stability of VFD process, and the consistency of the quality in the final products, for translating into a larger scale.

4 Conclusions

The use of VFD processing of fish oil for its encapsulation was compared with use of conventional homogenization. The diameter of encapsulated particles generated using homogenization ranged from 2 to 4 μm in diameter, whereas much smaller encapsulated particles were generated in the VFD, w/o/w spheroidal particles 50 to 250 nm in diameter, which potentially are more beneficial towards absorption of the fish oil. The VFD simplifies the procedure for water-in-oil-in-water (w/o/w) encapsulation, avoiding complicated and expensive processing, the use of organic solvents, and dramatically reduces the number of processing steps. Overall, the VFD provides a new direction for bottom–up facial and scalable processing with potential for food processing. Further studies on the use of the VFD in forming nano-meter dimension particles will focus on further systematically varying the

different operating parameters of the VFD (rotational speed, tilt angle, concentrations and ratio of components, temperature, and flow rates), the choice of phospholipid and combinations thereof, and the nature of the fish oil.

Acknowledgements:

The authors acknowledge support of this work by the Australian Research Council and the Australian Microscopy and Microanalysis Research Facility (AMMRF) and Australian National Fabrication Facility (ANFF) for SEM, dual target sputter coater and AFM imaging. Authors also acknowledge Adelaide Microscopy at The University of Adelaide, South Australia for TEM imaging.

References:

Adeyemi, S.A., Jon, R.C., Thomas, P., Paige, R. & Arturo, A.K. (2014). Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. *Environmental Science and Technology,* 48, 12561-12568.

Betoret, E., Betoret, N., Rocculi, P., & Rosa M. (2015). Strategies to improve food functionality: sctructure-property relationships on high pressures homogenization, vacuum impregnation and drying technologies. *Trends in Food Science & Technology*, 46, 1-12.

Britton, J. & Raston, L.C. (2017). Multi-step continuous-flow synthesis. Chemical Society Review, 46, 1250-1271.

311 Britton, J., Dyer, R.P., Majumdar, S., Raston, C.L. & Weiss, G.A. (2017). Ten-minute protein
312 purification and surface tethering for continuous-flow biocatalysis. Angewandte Chemie,
313 129, 2336-2341.
314
315 Britton, J., Meneghini, L.M., Raston, C.L. & Weiss, G.A. (2016). Accelerating enzymatic
316 catalysis using vortex fluidics. Angewandte Chemie, 55, 11387-11391.
317
318 Britton, J., Chalker, M.J., & Raston, L.C. (2015). Rapid vortex fluidics: continuous flow
319 synthesis of amides and local anesthetic lidocaine. *Chemistry-A European Journal*, 21,
320 10660-10665.
321
322 Britton, J., & Raston, L.C. (2014). Continuous flow vortex fluidic production of biodiesel. *RSC*
323 *Advances*, 4, 49850-49854.
324
325 Chen, X., Smith, N.M., Iyer, K.S., & Raston, C.L. (2014). Controlling nanomaterial synthesis,
326 chemical reactions and self assembly in dynamic thin films. Chemical Society Review, 5,
327 1387-1399.
328
329 Herrmann, J., & Bodmeier, R. (1995). Somatostatin containing biodegradable microspheres
330 prepared by a modified solvent evaporation method based on W/O/W-multiple emulsions.
331 *International Journal of Pharmaceutics*, 126, 129-138.
332

333 Honary, S., Ebrahimi, P., & Hadianamrei. (2014). Optimization of particle size and
334 encapsulation efficiency of vancomycin nanoparticles by response surface methodology.
335 *Pharmaceutical Development and Technology*, 19, 987-988.
336
337 Li, J., Shen, Z., Ma, X., Ren, W., Xiang, L., Gong, A., Xia, T., Guo, J. & Wu, A. (2015).
338 Neuropeptide YY1 receptors meditate targeted delivery of anticancer drug with
339 encapsulated nanoparticles to breast cancer cells with high selectivity and its potential for
340 breast cancer therapy. *ACS Applied Materials & Interface,* 7, 5574-5582.
341
342 Li, S., Tan, H., Wang, N., Zhang, Z., Lao, L., Wong, C., & Feng, Y. (2015). The role of oxidative
343 stress and antioxidants in liver disease. *International Journal of Molecular Sciences*. 16,
344 26087-26124
345
346 Meng, F.T., Guang, H.M., Wei, Q. & Zhi, G.S. (2003). W/O/W double emulsion technique
347 using ethyl acetate as organic solvent: effects of its diffusion rate on the characteristics of
348 microparticles. *Journal of Controlled Release*, 91, 407-416.
349
350 Mo, J., Eggers, P.K., Chen X., Ahamed, M.R.H., Becher, T., Lim, L.Y., & Raston, C.L. (2015).
351 Shear induced carboplatin binding within the cavity of phospholipid minic for increased
352 anticancer efficacy. Scientific Reports, 5, 10414.
353
354 Mostafa, N., Jean-Rene,A., Marc-Antoine, P. & Harivardhan, R.L. (2018). Comparison of high
355 pressure homogenization and stirred bead milling for the production of nano-crystalline
356 suspensions. *International Journal of Pharmaceutics*, 547, 61-71.

Nazar, M.R., Hafeezullah, K. & Shahzad, N (2010). Encapsulation and characterization of controlled release flurbiprofen loaded microspheres using beewswax as an encapsulating agent. *Journal of Materials Science: Materials in Medicine*, 21, 1621-1630.

Sacanna, S., & Philipase, A.P. (2006). Preparation and properties of monodisperse latex spheres with controlled magnetic moment for field-induced colloidal crystallization and chain formation. *Langmuir*, 22, 10209-10216.

Sitepu, E.K., Corbin, K., Luo, X., Pye, S.J., Tang, Y., Leterme, S.C., Heimann, K., Raston, C.L., & Zhang, W. (2018). Vortex fluidic mediated direct transesterification of wet microalgae biomass to biodiesel. *Bioresource Technology*, 266, 488-497.

Vimalanathan, K., Shrestha, R.G., Zhang, Z., Zou, J., Nakayama, T. & Rastion, C.L. (2017). Surfactant-free fabrication of fullerene C_{60} nanotubules under shear. Angewandte Chemie,129, 9518-8521.

Vimalanathan, K., Chen, X., & Raston, L.C. (2014). Shear induced fabrication of intertwined single walled carbon nanotube rings. *Chemical Communications*, 50, 11295-11298.

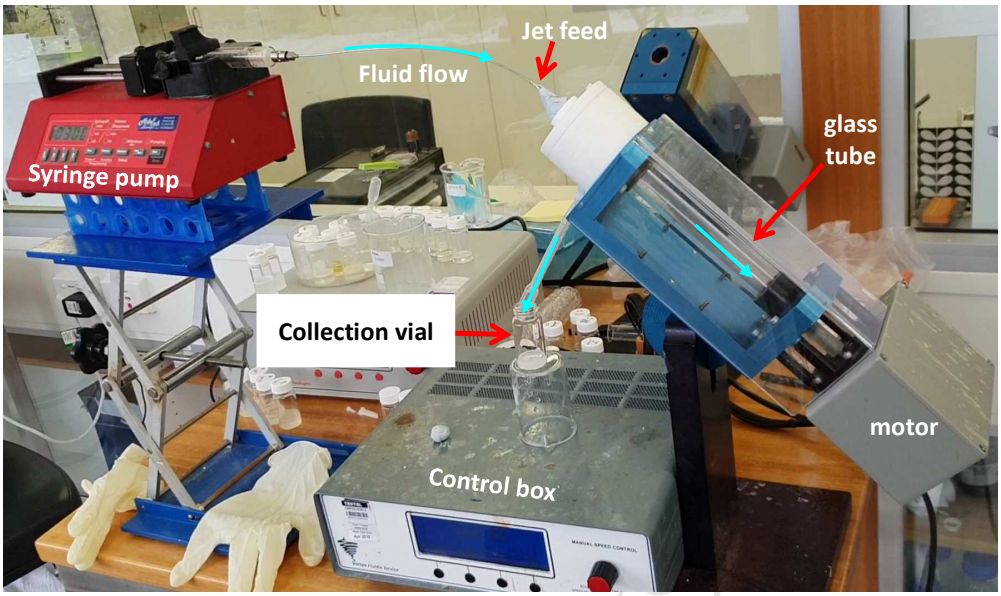


Figure 1: Photograph of the vortex fluidic device (VFD) highlighting its salient features.

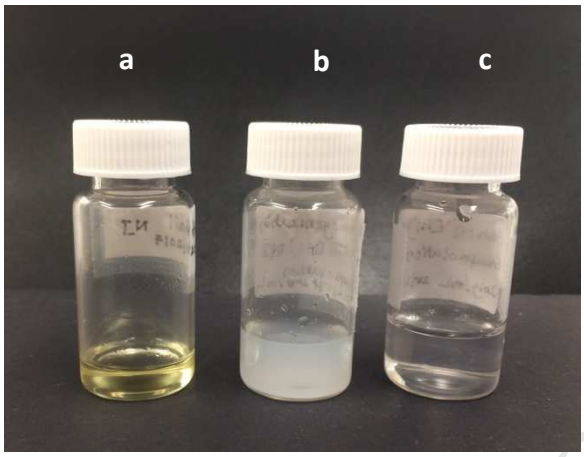


Figure 2: Photographs of different samples of fish oil: **(a)** As received fish oil. (**b)** Fish oil formulation processed using the homogenization method (speed: 13,500 rpm; time: 10 min; temperature: 25°C; sonication: 20 min; homogenizer: T25 digital ULTRA-TURRAX)**. (c)** Fish oil formulation processed using a vortex fluidic device (VFD) (dispersed phase: 2 g/100 mL, lipid/oil: 1/1 (w/w), speed: 8,000 rpm, flow-rate: 0.1 mL/min, tilt angle: 45^{o}, temperature: 25°C; sonication post VFD processing for 20 min).

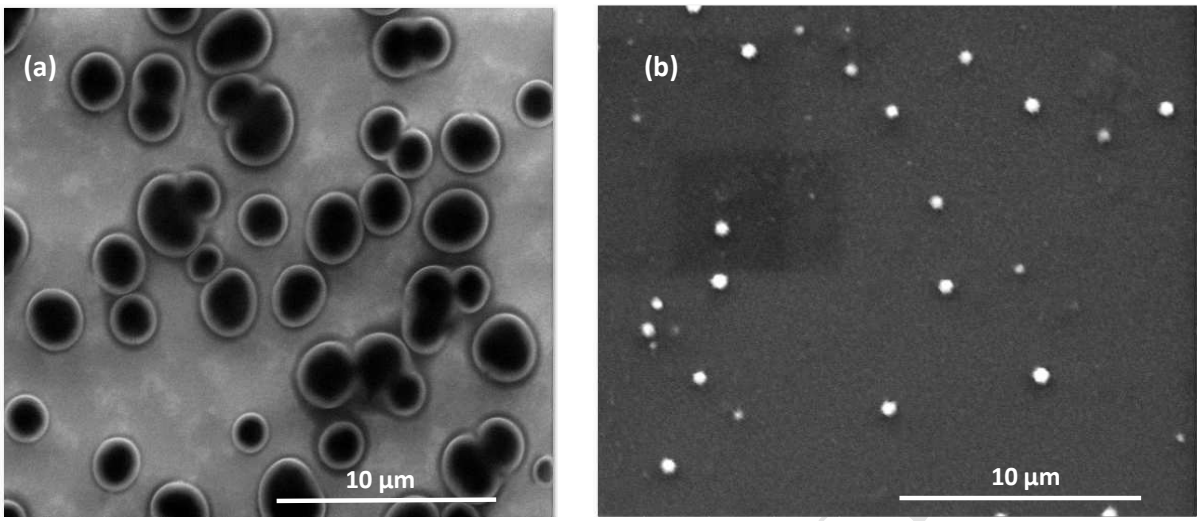
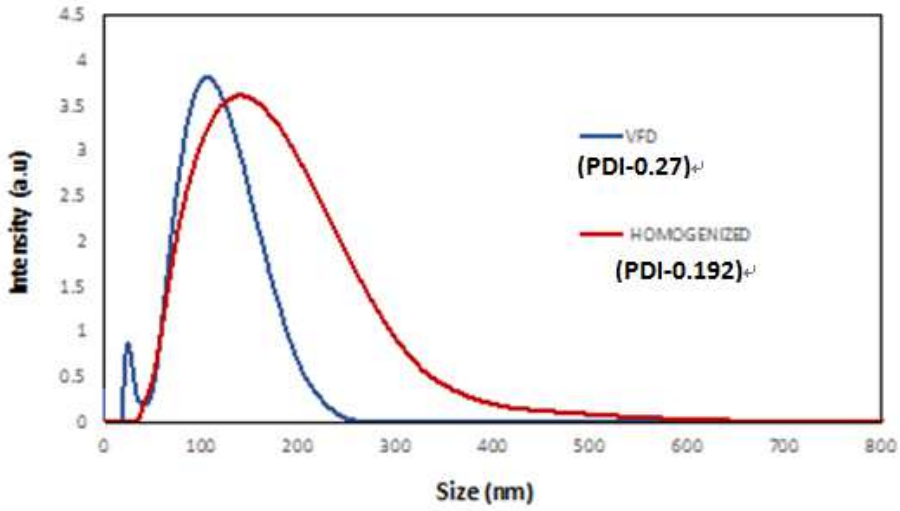


Figure 3: SEM images of fish oil encapsulation in phospholipids: **(a)** Generated using the homogenization method (speed: 13,500 rpm; time: 10 min; temperature: 25°C, sonication: 20 min). **(b)** Generated using VFD processing. Samples in (a) and (b) were coated with 2 nm Pt; dispersed phase: 2 g/100 mL, lipid/oil: 1/1 (w/w), speed: 8,000 rpm, flow-rate: 0.1 mL/min, tilt angle: 45^{o}, temperature: 25°C; sonication: 20 min.



PDI: Polydispersity

Figure 4: Dynamic light scattering (DLS) data (temperature 25^{o}C; He-Ne laser: 633 nm; detector angle: 173^{o}) for encapsulated particles produced using VFD processing, for the optimised processing parameters in Fig. 2 caption.

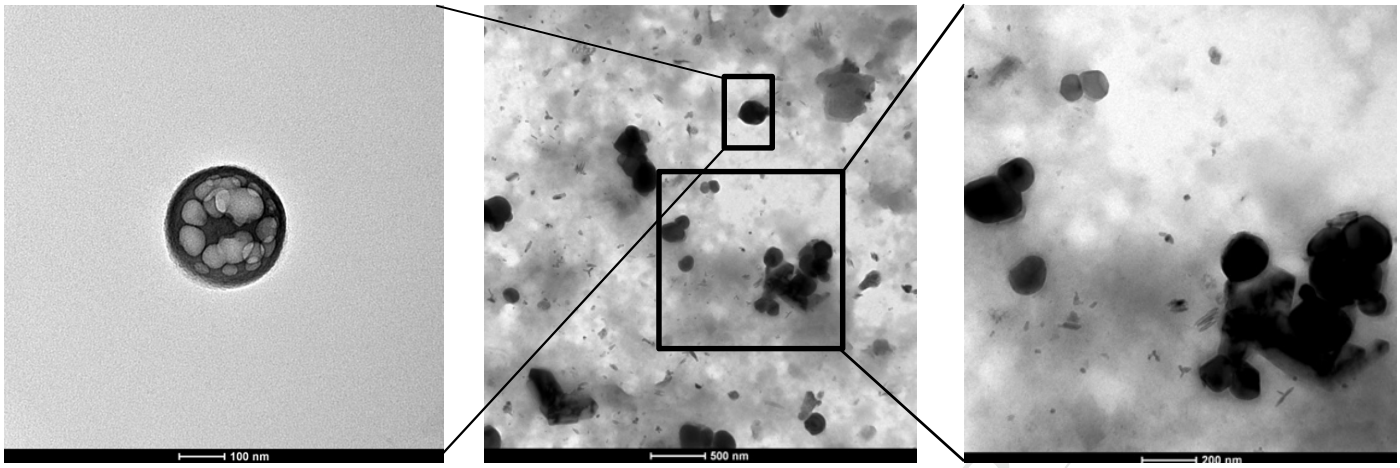


Figure 5: Transmission electron microscopy images of encapsulated particles produced using VFD processing under different magnification, for the optimised processing parameters in Figure 2. (Sample volume: 20 µL; staining: 2 g/100 mL uranyl acetate solution).

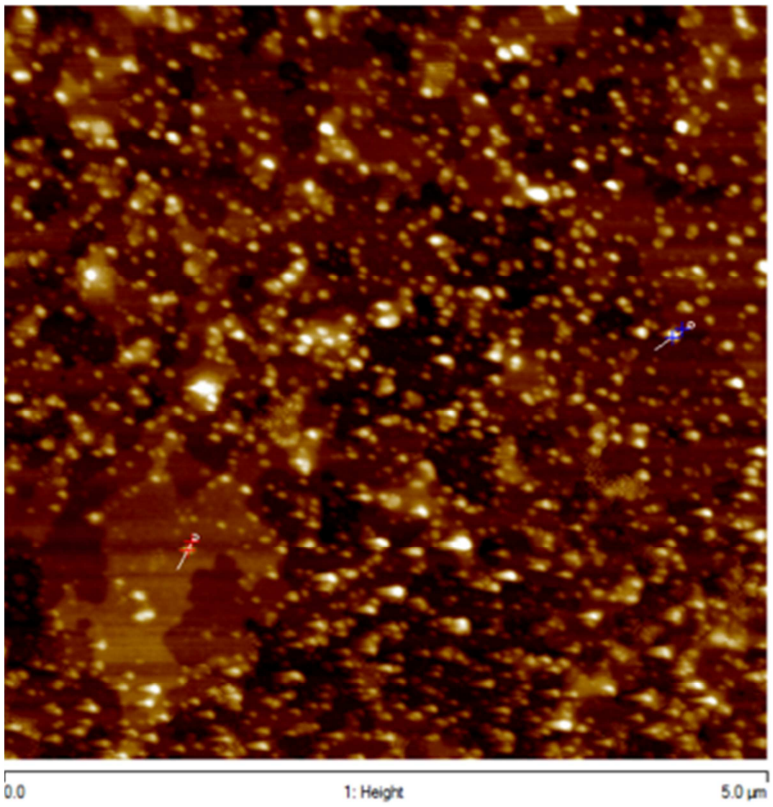


Figure 6: Atomic Force Microscopy image (sample volume: 20 µL, dispersed phase: 2 g/100 mL, lipid/oil: 1/1 (w/w), speed: 8,000 rpm, flow-rate: 0.1 mL/min, tilt angle: 45°, temperature: 25°C; sonication post VFD processing for 20 min.

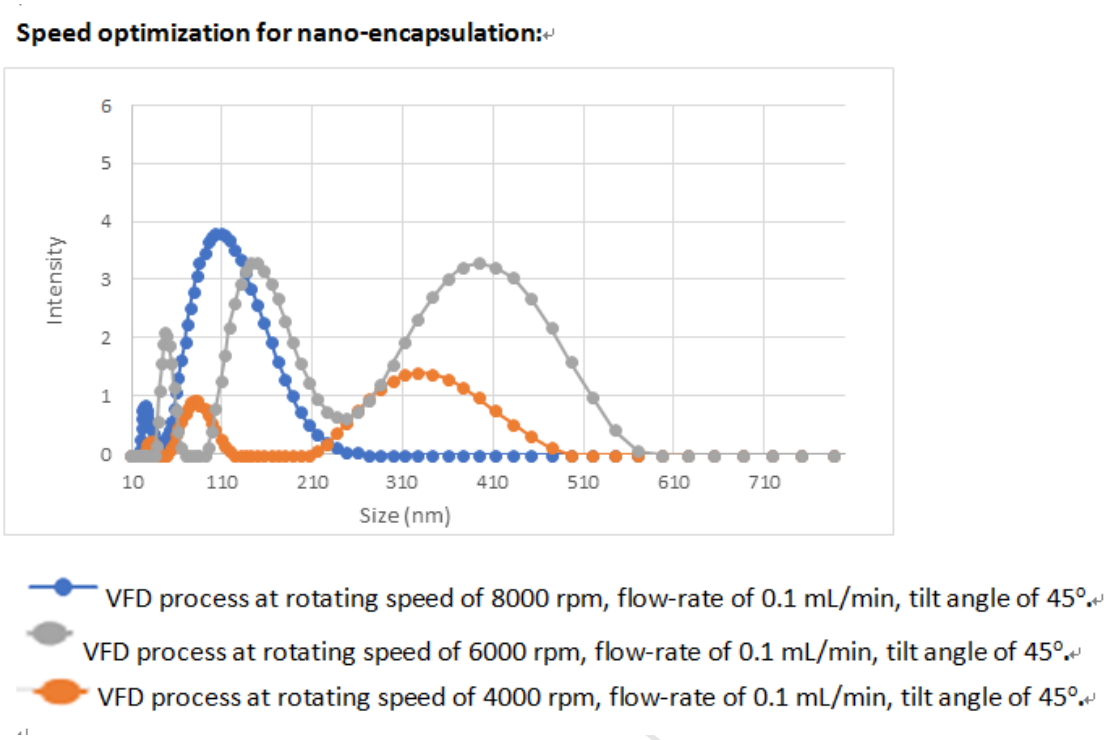
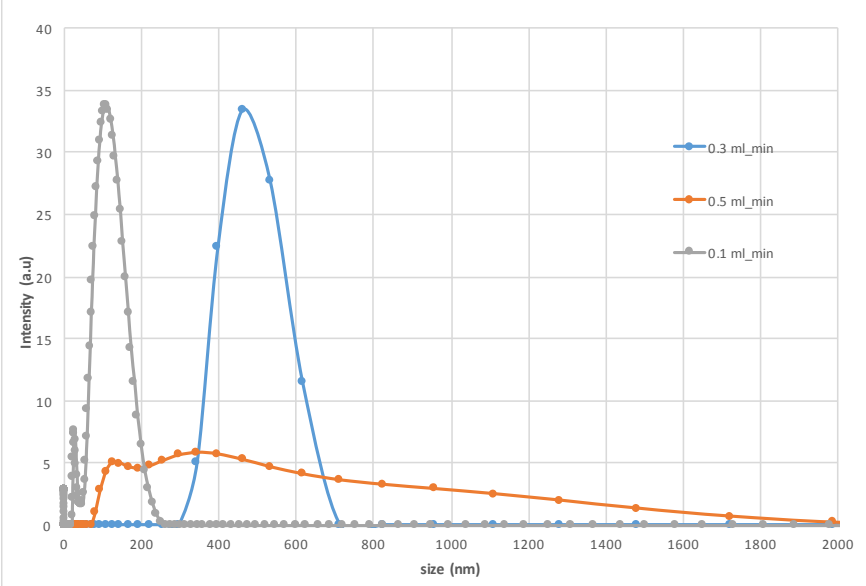


Figure S1. Dynamic light scattering (DLS) data for encapsulated particles generated using a vortex fluidic device (VFD) operating at different rotational speeds, with a fixed tilt angle and flow rate.

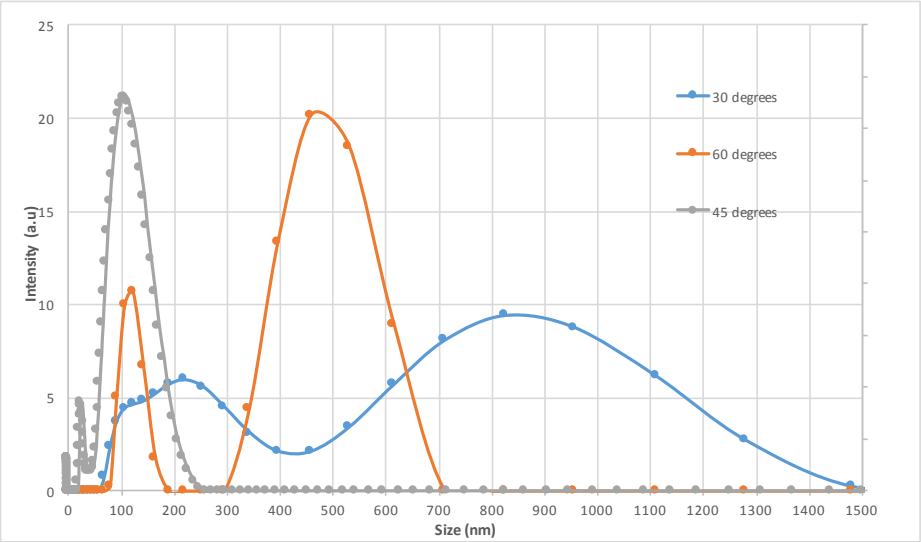


VFD process at rotating speed of 8000 rpm, flow-rate of 0.1 mL/min, tilt angle of 45°.

VFD process at rotating speed of 8000 rpm, flow-rate of 0.3 mL/min, tilt angle of 45°.

VFD process at rotating speed of 8000 rpm, flow-rate of 0.5 mL/min, tilt angle of 45°.

Figure S2. Dynamic light scattering (DLS) data for encapsulated particles generated using a vortex fluidic device (VFD) operating at different flow rate, with a fixed rotational speed and tilt angle.

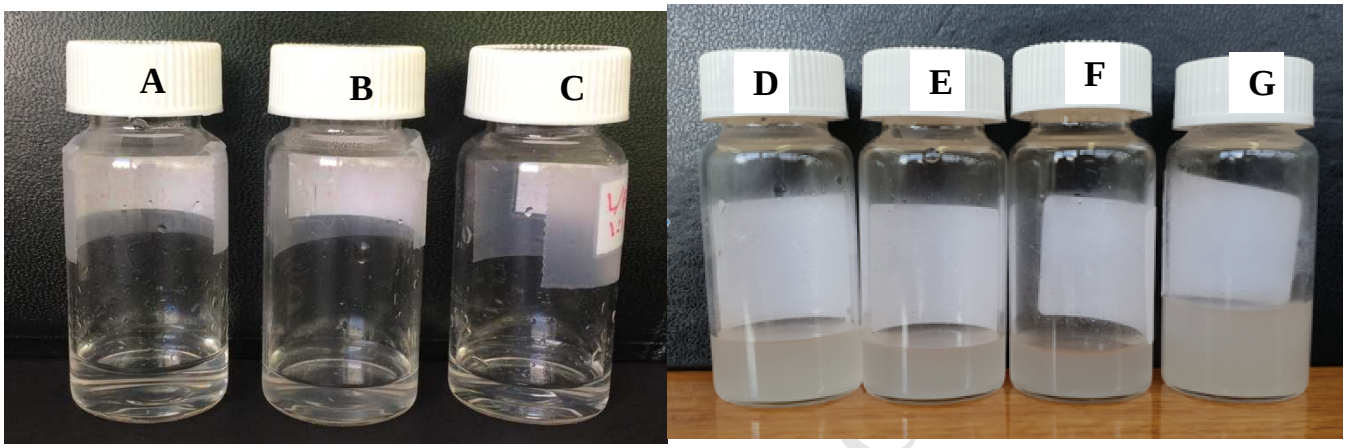


VFD process at rotating speed of 8000 rpm, flow-rate of 0.1 mL/min, tilt angle of 45°.

VFD process at rotating speed of 8000 rpm, flow-rate of 0.1 mL/min, tilt angle of 30°.

VFD process at rotating speed of 8000 rpm, flow-rate of 0.5 mL/min, tilt angle of 60°.

Figure S3. Dynamic light scattering (DLS) data for encapsulated particles generated using a vortex fluidic device (VFD) operating at different tilt angle, with a fixed tilt angle and different flow rates.



A**:** Rotational speed 4000 rpm, flow-rate 0.1 mL/min, tilt angle 45^{o}.
B: Rotational speed 6000 rpm, flow-rate 0.1 mL/min, tilt angle 45^{o}.
C: Rotational speed 8000 rpm, flow-rate 0.1 mL/min, tilt angle 45^{o}.
D: Rotational speed 8000 rpm, flow-rate 0.3 mL/min, tilt angle 45^{o}.
E: Rotational speed 8000 rpm, flow-rate 0.5 mL/min, tilt angle 45^{o}.
F: Rotational speed 8000 rpm, flow-rate 0.1 mL/min, tilt angle 30^{o}.
G: Rotational speed 8000 rpm, flow-rate 0.1 mL/min, tilt angle 60^{o}.

Figure S4: Physical appearance of solutions after vortex fluidic device (VFD) processing under different operating parameters.

→Vortex Fluidic Device reduced the fish oil encapsulation diameter from μm level to nm level.

→Vortex Fluidic Device simplified water-in-oil-in-water encapsulation into one-step process.

→Vortex Fluidic Device provides a new encapsulation approach for a bottom-up approach.